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International Journal of Vaccines & Vaccination

Research Article

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Assessment of embryo fetal developmental toxicity study of hepatitis B (rDNA) vaccine in wistar rats

Abstract

Hepatitis B infection is one of the most deadly diseases causing acute as well as chronic infection to liver which globally affects 5% of people worldwide. A recently developed Hepatitis B vaccine by recombinant DNA technology has been shown to be potentially efficacious in prevention of Hepatitis B virus (HBV) mediated infection. The purpose of current study was to detect the adverse effects of the vaccine on the pregnant female rats and developing embryo/fetus during organogenesis exposure. If any. In addition, anti-HBV antibodies in pregnant females were measured during the study. Three groups of 25 females injected intramuscularly Hepatitis B (rDNA) vaccine at the dose level of 0.25, 0.5 and 1.0mL per animal once prior to cohabitation and once during gestation (day 10). Concurrent placebo control group was maintained to differentiate the effects of placebo from vaccine related effects. All females were mated to males of same stock with mating ratio of 2:1. The pregnant females were C-section about one day prior to delivery i.e. GD 20 to evaluate the uterine contents and the fetuses for external, visceral and skeletal anomalies. There was no abortion or death during the study. Expected local effects like mid swelling at injection site was observed which was attributed to common placebo related non-adverse effect. Gravimetric parameters did not reveal evidence of vaccine related toxicity. Pre natal parameters were comparable to control. There was no evidence of prenatal developmental toxicity and based on the results Hepatitis B (r-DNA) vaccine was not a teratogenic during the study. Immunogenicity profile showed measurable antibody titer that supports the use of the vaccine in the targeted human population.

Keywords: hepatitis b vaccine, immunogenicity, teratogenicity

Volume 3 Issue 3 - 2016

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Received: May 20, 2016 | **Published:** December 30, 2016

Introduction

Hepatitis B infection is one of the most deadly diseases causing acute as well as chronic infection to liver which globally affects 5 % of people worldwide. As no specific treatment is available, greatest emphasis is placed on prevention through immunization. Hepatitis B virus (HBV), a member of the hepadnaviridae family, is an envelope, circular, single-stranded, and partially double-stranded DNA virus.[1–3] The virus interferes with the functions of the liver while replicating in hepatocytes. A recently developed Hepatitis B vaccine is produced by r-DNA technology where *Hansunela Polymorpha* cells containing gene for Hepatitis B surface antigen are grown to achieve maximum growth and then lysed to recover recombinant protein. It is being further purified, where it form like noninfectious virus-like particles (VLPs) which are immunogenic. They are formulated with alum hydroxide as final vaccine in liquid form. The safety of the Hepatitis B (r-DNA) vaccine has been evaluated in nonclinical and clinical studies.

Because the Hepatitis B (r-DNA) vaccine would be indicated for women of child bearing potential, a nonclinical developmental and reproductive toxicity study was required to support the clinical development. The primary purpose of this study is to detect the potential adverse effects of the Hepatitis B (r-DNA) vaccine on the pregnant female rats and developing embryo/fetus. Limited data indicate no apparent risk of adverse events to the mother or the developing fetus when Hepatitis B vaccine is administered to pregnant women. Information from this study may be used to assess the risk potential, if the target population for the vaccine includes women of child bearing potential.

The study was designed based on a guidance document from the World Health Organization titled "Nonclinical evaluation of vaccine, WHO technical report series no.927, 2005. Additionally, immunogenicity was evaluated in the animal species chosen for the developmental and toxicology assessment, the Wistar rat to confirm that the vaccine was immunogenic in pregnant Wistar rats. The results of the immunogenicity study and the developmental and reproductive toxicology study are reported here.

Materials and methods

Animals, husbandry and study design

Hundred adult female rats (8weeks of age) of the Wistar strain (Zydus Research Centre, Ahmedabad, India), were selected for this experiment. Animals were maintained under standard laboratory conditions (Lighting: 12 / 12hour, Temperature: 21-26°C, Relative Humidity: 33 to 58%) with certified rodent pellet feed (Harlan Teklad® T- 2018) and drinking water filtered *ad libitum*. During mating, one male was housed with up to two females and after evidence of mating; each female housed individually. Autoclaved corn cob was used as bedding material. Animals were divided equally twenty five per groups into placebo (1.0mL/animal-GI), low (0.25mL/animal-GII), mid (0.5mL/animal-GIII) and high (1.0mL/animal-GIV) dose groups. The animals received the vaccine and placebo by intramuscular injection administration at a volume of 0.2mL per site in per rat. Animal were dosed into the anterior thigh (Quadriceps muscle). Hepatitis B (r-DNA) vaccine formulation has been administered to female rats once prior to mating and once during pregnancy (gestation day10). Concurrently the control group animals were treated with placebo alone for the same duration. Test item and placebo were received from Vaccine Technology Centre, Cadila health Care Ltd. The test materials were stored in a refrigerator (2-8°C), protected from light. Each 1.0mL vial contains 27.5µg Purified Hepatitis B Surface antigens.

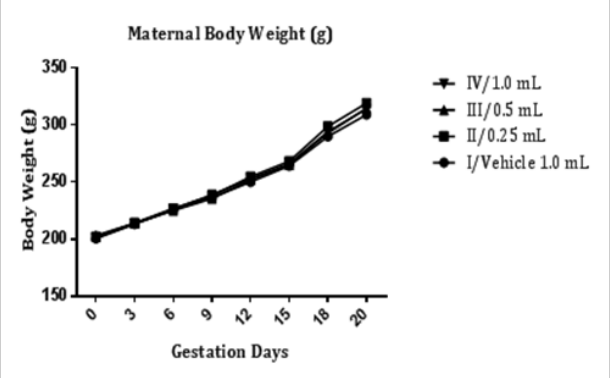

Figure 1 Maternal Body weight (g).

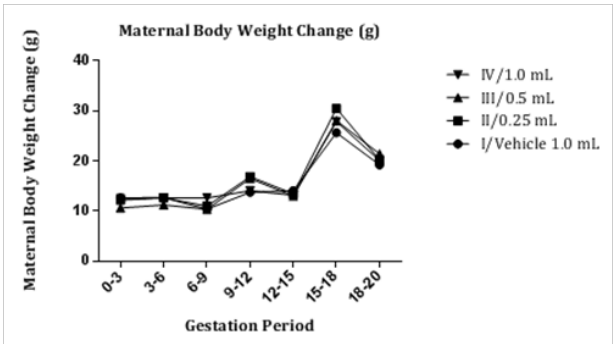

Figure 2 Maternal Body weight Change (g).

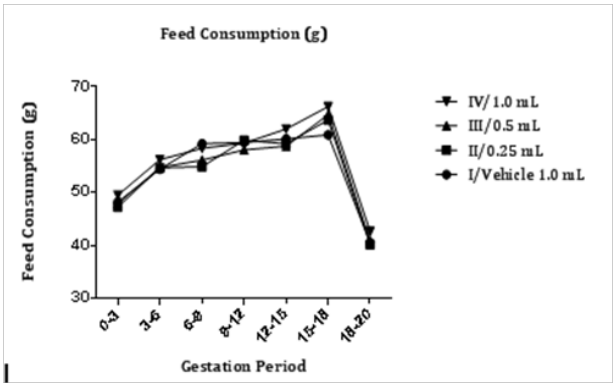

Figure 3 Feed Consumption (g).

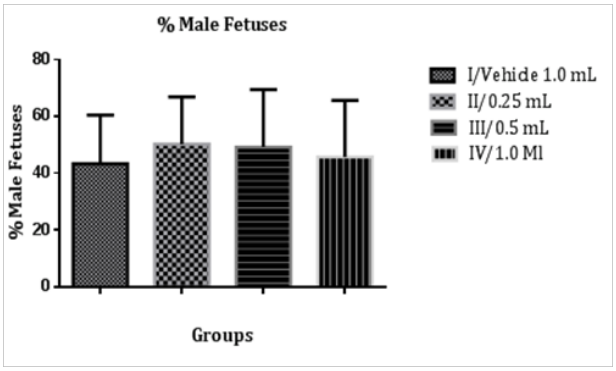

Figure 4 Percentage Male Fetuses.

Females were weighed on presumed gestation day 0, 3, 6, 9, 12, 15, 18 and 20 (terminal sacrifice) during gestation period. Body weight change was calculated for the period 0-3, 3-6, 6-9, 9-12, 12-15, 15-18, 18-20 and in total from day 0-20 during gestation. Feed input and leftover was recorded on respective day of weighing and feed consumption was calculated for period of 0-3, 3-6, 6-9, 9-12, 12-15, 15-18 and 18-20 during gestation and pregnant females were sacrificed about one day prior to expected date of parturition (on gestation day 20) by carbon dioxide asphyxiation. All animals retained on study were subject to a detailed necropsy at termination. Gross lesions, was collected and preserved in 10% neutral buffered formalin for further histopathological examination. Each female, the reproductive tract, complete with ovaries, was dissected out. The numbers of corpora lutea (assessed for each ovary before removal), implantation sites, resorption sites (classified as early or late), live and dead fetuses were recorded. Each fetus was weighed, sexed and examined for external abnormalities. The absence of implantation sites in apparently non-pregnant females was confirmed by 10% ammonium sulfide staining. Fetuses were humanely euthanized before evisceration and/or fixation. After external examination 50% litters were subjected to fresh visceral examination for soft tissue alteration and other 50% litters were eviscerated before processing and staining with Alizarin red for skeletal examination. The fetuses which were viscerally examined were also evaluated for head razor anomalies.

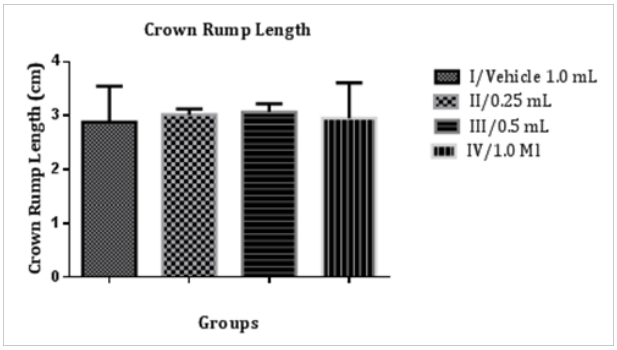

Figure 5 Crown Rump Length (cm).

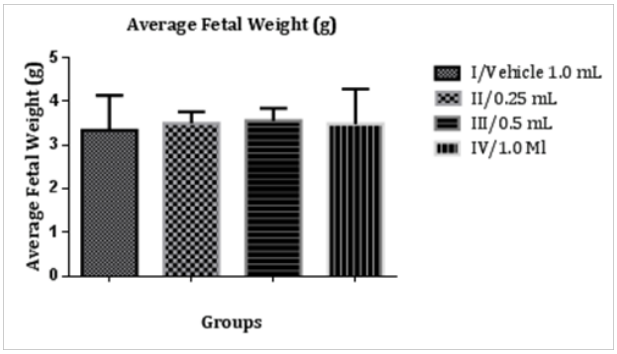

Figure 6 Averages Fetal Body Weight (g).

Blood samples were taken from the retro-orbital plexus under isoflurane anesthesia on pre and post treatment (at scheduled terminal sacrifice). All samples were collected without anticoagulant and serum was separated after centrifugation at approximately 4000rpm for 10min. Serum sample were frozen (approximately -70°C) until the antibody analysis was performed.

Results and Discussion

The present study was conducted to investigate the potential embryo-fetal toxicity of Hepatitis B (r-DNA) vaccine in Wistar rats. Since the target population for the vaccine includes women of childbearing potential, the potential effects of Hepatitis B (r-DNA)

vaccine administration during organogenesis was investigated in a pre-clinical model.

All pregnant females from low, mid and high dose groups survived to their scheduled termination. Daily clinical observations during the gestation period did not reveal any adverse clinical sign in the dams amongst the treated and control groups. Clinical sign like transient mild swelling at site of injection was observed on day 2 and 3 after treatment and on gestation day 11 and 12 in females treated with placebo and vaccine at dose 1.0mL/animal and it was considered as expected vehicle (placebo) related non-adverse finding.[4,5]

The maternal body weight, body weight changes and feed intake during gestation period was found to be normal in all the groups and was comparable with control group (Figure1-3). The pregnancy data such as number of females pregnant at term, percent pregnancy rate, no. of females with viable fetuses were found to comparable amongst treatment and control groups (Table 1).

Intramuscular administration of Hepatitis B (r-DNA) Vaccine did not revealed any adverse effect on gravid uterus weight, corpora lutea count, number of implantation sites, live and dead conceptuses and early and late resorptions were found to be comparable with control. The derived uterine data like corrected maternal body weight, relative uterus weight, pre and post implantation loss and implantation index were not significantly altered up to 1.0mL/animal (Table 2).

No significant differences in litter data like total no. of fetuses, no. of male and female fetuses and sex ratio (% male fetuses) were seen up to 1.0 mL/animal (Figure 4). The absolute total fetal body weight was statistically significant in low dose as compared to control group while mid and high dose group revealed no significance. Fetal parameters like absolute fetal body weight (male and female), crown-rump length (Figure 5) and average fetal body weight (Figure 6) did not reveal any treatment related adverse effect and were found to be comparable with control group.

Table 1 Pregnancy Data

Parameters	Group	I	II	III	IV
	Dose (mL/Animal)	Vehicle (1.0)	0.25	0.5	1
No. of Females used		25	25	25	25
No. of Females Mated		25	25	25	25
No. of Pregnant Females at Term		21	21	19	22
No. of Non-pregnant Females		4	4	6	3
Pregnancy Rate (%)		84	84	76	88
No. of Females with all Viable fetuses		17	21	18	16
Females with all Viable fetuses (%)		80.95	100	94.74	72.73
No. of females with Resorptions		4	0	1	6
Females with Resorptions (%)		19.05	0	5.26	27.27

Table 2 Uterine Data

Observation	Group and Dose (mL/animal)			
	I	II	III	IV
	Vehicle (1.0)	0.25	0.5	1
Gravid Uterus Weight with cervix and ovaries (g)	56.998	65.575	59.907	58.302
No. of Corpora lutea	11.95	12.71	12	11.77
Total No. of Implants	10.86	12.19	11.05	11
No. of Live Implants	10.43	12.19	11	10.45
No. of Dead Implants	0	0	0	0
Total No. of Resorption (Early + Late)	0.43	0	0.05	0.55
Implantation Loss %				
Pre	9.39	4.17	10.43	6.99
Post	6.09	0	0.48	7.05

Table 3 Fetus Goss External Examination

Group	I	II	III	IV
Dose (mL/animal)	Vehicle (1.0)	0.25	0.5	1
No. of Fetuses/litter	219/20	256/21	209/19	230/21
Small	4-Jan	4-Aug	4-Jun	2-Feb
Anasarca	0/0	0/0	1-Jan	0/0
Domed	1-Feb	0/0	2-Feb	0/0
Petechial haemorrhage	2-Feb	0/0	0/0	0/0

An external abnormality (Table 3) such as small size fetus, anasarca, domed head and petechial haemorrhage in head were observed in control and treated group and all observation was found to be within in-house historical control range from 13 embryo fetal studies performed between 2008 to 2015 (Not-Published). In addition, it was not dose-related and therefore, it was considered to be an incidental finding.[6] An increase in the incidence of fetal visceral ureter kinked and or dilated, adrenal hemorrhage and lateral and third ventricle was observed in control group; however it was found in within in-house historical range (Table 4 & 5). An increase in the incidence of fetal skeletal variations (Table 6) observed in treated groups occurred in a non-dose dependent manner. Therefore it was not considered to be treatment-related. The occurrences of fetal skeletal abnormalities compared well between the groups. They were within the normal historical range and they are therefore considered to be of a spontaneous nature and not vaccine related toxicological significance.[4–6]

Citation: Patel SR, Patani K, Jain P, et al. Assessment of embryo fetal developmental toxicity study of hepatitis B (rDNA) vaccine in wistar rats. *Int J Vaccines Vaccin.* 2016;3(3):11–12. DOI: 10.15406/ijvv.2016.03.00069

Table 4 Fetus Visceral Examination

Group	I	II	III	IV
Dose (mL/Animal)	**Vehicle (1.0)**	**0.25**	**0.5**	**1**
No. of Fetuses/litter	105/20	123/21	99/18	110/21
Adrenal				
Adrenal (R): haemorrhagic	1-Jan	0/0	0/0	0/0
Ureter				
Ureter (L): kinked	4-Apr	0*/0	1-Jan	0/0*
Ureter (R): kinked	2-Feb	1-Jan	2-Feb	2-Feb
Ureter (R): convoluted	3-Mar	1-Jan	0/0	1-Jan
Ureter (R): dilated	1-Jan	0/0	1-Jan	0/0
Ureter (L): convoluted	0/0	1-Jan	0/0	1-Jan
Ureter (B): dilated	0/0	0/0	1-Jan	0/0
Ureter (L): slightly dilated	0/0	0/0	1-Jan	0/0
Ureter (B): kinked	0/0	0/0	1-Jan	1-Jan

Table 5 Fetus Head Razor Examination

Group	I	II	III	IV
Dose (mL/Animal)	**Vehicle (1.0)**	**0.25**	**0.5**	**1**
No. of Fetuses/litter	105/20	123/21	99/18	110/21
Ventricles				
Lateral Ventricles: Dilated	3-Mar	1-Jan	3-Mar	2-Feb
Lateral Ventricles: Slight Dilated	2-Feb	3-Apr	1-Jan	3-Mar
Third ventricle: Slight Dilated	2-Feb	1-Jan	2-Feb	2-Feb

Table 6 Fetus Skeletal Examination

Group	I	II	III	IV
Dose (mL/Animal)	**Vehicle (1.0)**	**0.25**	**0.5**	**1**
No. of Fetuses/litter	114/20	133/21	110/19	120/21
Skull				
Frontal- Incomplete Ossification	2-Mar	0/0	1-Jan	1-Jan
Parietal-Incomplete Ossification	27/10	16 */9	28/12	18/11
Interparietal-Incomplete Ossification	22/13	17 /9	17/9	14 /12
Supra occipital-Incomplete Ossification	16/9	8-Nov	4 **/3	3 **/3
Zygomatic arch-Incomplete Ossification	5-Aug	3-Mar	2-Feb	2-Mar
Ribs				
14th Rib- Extra Ossification Center	28/12	22/15	12 **/10	15 */12
14th Rib- Short supernumerary	7-Aug	3-Jun	3-Jun	2-Mar
Rib-Wavy	4-May	0 */0*	3-May	0*/0 *
Sternebrae				
1st sternebra- Unossified	0/0	0/0	1-Feb	0/0
1st sternebra- Incomplete Ossification	0/0	1-Jan	0/0	0/0
2nd sternebra- Dumbbell Ossification	0/0	1-Jan	0/0	0/0
2nd sternebra- Misshapen	1-Jan	0/0	0/0	0/0
2nd sternebra- Unossified	0/0	0/0	2-Mar	0/0
3rd sternebra- Unossified	0/0	0/0	2-Mar	0/0
3rd sternebra- Misshapen	1-Jan	2-Feb	1-Jan	0/0
4th sternebra- Misshapen	6-Jun	2-Feb	3-Mar	1-Jan
4th sternebra- Misaligned	0/0	1-Jan	0/0	0/0
4th sternebra- Unossified	0/0	2-Feb	2-Mar	0/0
5th sternebra- Misaligned	0/0	1-Jan	0/0	0/0
5th sternebra- Incomplete ossification	7-Sep	7-Sep	7-Nov	7-Aug
5th sternebra- Bipartite	1-Jan	0/0	0/0	0/0
5th sternebra- Unossified	3-Jun	14/7	18**/10	17*/10
5th sternebra- Misshapen	2-Feb	1-Jan	1-Jan	1-Jan
6th sternebra- Incomplete ossification	8-Aug	9-Nov	3-Jul	6-Jun
6th sternebra- Unossified	3-Apr	2-Mar	6-Sep	5-May
Thoracic Centrum				
9th Thoracic centrum-Dumbbell Ossification	1-Jan	0/0	0/0	0/0
9th Thoracic centrum-Bipartite	0/0	1-Jan	0/0	0/0

Citation: Patel SR, Patani K, Jain P, et al. Assessment of embryo fetal developmental toxicity study of hepatitis B (rDNA) vaccine in wistar rats. *Int J Vaccines Vaccin.* 2016;3(3):11–12. DOI: 10.15406/ijvv.2016.03.00069

Table Continued...

Group	I	II	III	IV
Dose (mL/Animal)	**Vehicle (1.0)**	**0.25**	**0.5**	**1**
10th Thoracic centrum-Bipartite	1-Jan	1-Jan	0/0	1-Jan
10th Thoracic centrum-Dumbbell Ossification	1-Jan	1-Jan	0/0	1-Jan
10th Thoracic centrum-Incomplete Ossification	0/0	1-Jan	0/0	0/0
11th Thoracic centrum-Dumbbell Ossification	7-Jul	2-Feb	4-Apr	6-Jul
11th Thoracic centrum-Bipartite	0/0	0/0	1-Jan	1-Jan
12th Thoracic centrum-Bipartite	1-Jan	2-Feb	0/0	3-Mar
12th Thoracic centrum-Dumbbell Ossification	2-Feb	2-Feb	2-Feb	2-Feb
13th Thoracic centrum-Asymmetric Ossification	1-Jan	0/0	0/0	0/0
13th Thoracic centrum-Bipartite	1-Feb	1-Jan	1-Jan	1-Jan
13th Thoracic centrum-Dumbbell Ossification	2-Mar	2-Feb	1-Jan	0/0
Meta carpal- Unossified	0/0	0/0	0/0	1-Jan
Pubis- Incomplete Ossification	2-Feb	2-Feb	1-Feb	0/0
Pubis- Unossified	1-Jan	0/0	0/0	0/0

The placenta was found to be normal. White deposits and red discoloration was observed at site of injection during necropsy examination in animals from placebo and test item treated groups. Histopathological examination of gross lesions at the site of injection where observed minimal to mild chronic inflammation and muscle necrosis, minimal hemorrhage which was considered as expected vehicle related non-adverse findings as these lesions were only restricted to the injection site.[7] The study result reveals that the Hepatitis B (r-DNA) vaccine is immunogenic in pregnant female rats by producing antibodies which are measurable up to the 1/500, 1/10000 and 1/30000 for low, mid and high dose respectively. The immunogenicity profile showed measurable antibody titer for Hepatitis B (rDNA) vaccine at all vaccine treated dose groups treated in pregnant rats. There was no maternal or developmental toxicity in the Hepatitis B (r-DNA) vaccine treated group.

Conclusion

In conclusion, under the conditions of these studies, intramuscular administration of the Hepatitis B (r-DNA) vaccine, formulated with an aluminum adjuvant was well-tolerated in pregnant female Wistar rats during organogenesis period and was non-teratogenic in Wistar rats.

Acknowledgments

The authors would like to acknowledge the excellent technical support that made this complex study possible and those colleagues who helped with the review of this manuscript and their very helpful comments. I would also like to thank to Zydus Research Center, Ahmedabad, Gujarat, India, for providing resources at research facility.

Conflicts of interest

Author declares there are no conflicts of interest.

Funding

None.

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